PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	John A. Stolk et al.
Application No.	:	09/970,966 Confirmation No.: 3194
Filed	:	October 2, 2001
For	:	COMPOSITIONS AND METHODS FOR THE THERAPY AND DIAGNOSIS OF OVARIAN CANCER
Examiner	:	Mary K. Zeman
Art Unit	:	1631
Docket No.	:	484C6
Date	:	September 3, 2003

Examiner: Mary K. Zeman

Via Facsimile: (703) 872-9306

DECLARATION OF RAYMOND L. HOUGHTON, Ph.D., UNDER 37 C.F.R. § 1.132

The undersigned, Dr. Raymond L. Houghton, hereby declares:

1. I am a Scientist at Corixa Corporation the assignee of the subject application. The following experiments were carried out under my supervision.

2. I have reviewed the Office Action dated May 5, 2003 in the subject application, including the rejections under 35 U.S.C. §112, and provide this Declaration for the purpose of providing more evidence to the Examiner that the SEQ ID NOS:199 and 214, can be used to diagnose the presence of ovarian cancer in a sample suspected of containing ovarian cancer.

3. I am familiar with the teachings of the above referenced United States Patent application.

3. Following the teachings of the above referenced application; samples were taken from subjects without cancer, subjects with ovarian cancer, and subjects with a cancer other than ovarian cancer and were screened using primers specific for the tumor antigen known as O591S. O591S is described in the above reference patent application as SEQ ID NOS 199 and 214. The presence of elevated levels of O591S in a sample corresponded to a positive diagnosis for ovarian cancer. Elevated levels of O591S transcripts were not found in the samples from normal patients or the patients with a cancer other than ovarian cancer. The results of these screenings are given in more detail in paragraphs 4 and 5.

4. Primers specific for O591S were used to detect circulating tumor cells in patients with cancer. 10 ml of peripheral blood samples were collected in EDTA from 11 normal female donors (D472-D426) and 12 ovarian cancer patients. Blood was treated with RosetteSep™ Tumor Cell Enrich antibody cocktail (StemCell Technologies, Vancouver) and mononuclear cells were collected using a density gradient (Sigma Accuspin System-Histopaque-1077). mRNA was isolated (Roche mRNA isolation kit) and reverse transcribed into cDNA using Oligo-dT primers and Superscript Reverse Transcripase (Gibco). The O591S transcript was amplified using SYBR® Green PCR core reagents (ABI) and following primer pairs: FWD-5'ctgctctccgtagccttcatgt and REV-5'ccagcgagcccttctgg. An additional primer and probe pair, designed to span intron-exon borders to exclude genomic DNA amplification, was also tested, O591S-IS3-F-5'tgctaccagtgtgaagaattcca, O591S-IS3-R-5'gtacatgatcccggcacttt, O591-IS3-probe-5'ccccgagttcattgtgaattgcacg. Expression levels for each gene were measured by quantitative real-time PCR using the ABI 7700 Prism™ sequence detection system (Applied Biosystems, Foster City, CA). PCR conditions were one cycle at 50° for 2 min, one cycle at 95° for 10 min, 95° for 15'' and 60° for 1' for 50 cycles. O591S showed elevated expression signal in blood samples of 4 ovarian cancer patients. All four patients had recurrent metastatic disease. O591S expression signal was also detected using the intron-spanning primer pair (IS3) in two samples. The expression signal was negative in the samples obtained from the normal patients.

5. Primers specific for O591S were used to detect ovarian cancer in patient ascite fluids (circulating tumor cells). Cells were collected from ascitic fluids of patients with ovarian cancer, and total RNA was isolated using TRIZOL™. Real-time RT-PCR was used to detect expression

of O591 (SYBR® Green PCR core reagents, ABI) with following primer pairs: O591-FWD: 5'aagtgccgggatcatgtacc andO591-REV: 5'gtaccccggcagaggcgat. High O591S expression signals (>500 copies) were detected in 9 out of 18 ovarian cancer but not in 5 non-cancer, 1 benign ovary and 3 other malignancy (colon cancer, cervical cancer, small bowel cancer) ascites samples.

4. The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements, and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

Raymond L. Houghton, Ph.D.

9/3/03
Date